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DOES *MYZUS PERCISEAE* REDUCE *BOTRYTIS CINEREA* INFECTION?

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ABSTRACT
Direct and indirect interactions are the consequences that occur following attack on plants and their produce by pathogens and insect herbivores. Here experiment was presented which shows that bi-directional interaction occur when systemic pathogen *Botrytis cinerea* Pears Fr (Helotiales Sclerotiniaceae) and an insect herbivore green peach aphid *Myzus persicae* share same host plant lettuce *Lactuca sativa* (Asteraceae: Compositae). The bi-directional interaction result in reduction of *B. cinerea* lesion when compared with the infected but uninfested plants. Result also showed that infected uninfested plants showed reduced leaf number, internode length, fresh and dry weight of the plant than *B. cinerea* infected plants which were infested with *M. persicae*.

Keywords: *Botrytis cinerea,* Bi-directional Interaction, Growth of Lettuce, *Myzus persicae.*

INTRODUCTION
The world-wide demand for crop plants and their products for food, feed and fuel is increasing dramatically (Oerke, 2006). However, crop plants are at risk and subject to attack continuously by a wide range of pathogens (Elad and Steward 2004; Oerke, 2006; Fatima, 2010). Plants show various strategies to defend against attack by pathogenic fungi by activating direct and/or indirect defense mechanisms (Stout *et al*. 2006; Fatima, 2010).

Interactions between herbivores and pathogenic fungi are possible as they can share the same host plant. Interactions may be direct, for example when the herbivore benefits from fungal pre-digestion of complex sugars, or simply by ingesting the fungus itself (Ohgushi, 2005; Stout *et al.* 2006). Indirect interactions may occur where the pathogenic fungi spatially or temporarily changes the genetic make-up of the host plant in such a way that the host plant

becomes either more or less susceptible to the insect herbivore (Stout *et al.* 2006). The insect green peach aphid *Myzus persicae* and *Botrytis cinerea* a pathogenic fungus are of great economic importance due to their ability of spreading diseases causing wastage of plant and its produce resulting in serious economic losses (James, 1974; Agrios, 2005). The ability of *Botrytis cinerea* and *Myzus persicae* to share same host plant enables them to exhibit a negative interaction.

The pathogen *Botrytis cinerea* Pers. is a polyphagous and ubiquitous fungus that causes economically important diseases in a wide range of host species throughout the world. Greenhouse crops, field vegetables stored and transported fruits, small fruits ornamental flowers and bulbs, as well as forest seedlings are all attacked by the pathogen (Elad and Steward 2004). The species is pathogenic to a range of over 230 host plants species, including Angiosperms, Gymnosperms, Pteridophytes and Bryophyte and is saprophytic on senescent and dead plants tissues (Elad and Steward 2004).

The pathogen exists in nature as mycelia, conidiophores, conidia apothecia, ascospores, spermatia, germ-tubes, aprressoria and chlamydospores (Holz *et al.* 2004). Ascospores, conidia, mycelia and sclerotia are in various instances important forms of inoculum of disposal propagules although sclerotia and mycelia in host residues are the primary survival structures of *B. cinerea* (Elad and Steward 2004; Holz *et al.* 2004). The conidia are ubiquitous in the air and can be transported by wind or insects over long distance before infecting the next host (Agrios *et al.* 2005). Conidia can also be produced in large quantities in infected crops and can be dispersed over long distances (Jarvis, 1977).

The aphid *M. persicae* has a life cycle, which involves a regular movement between primary host (deciduous woody plants at the beginning and end of the season) and the secondary host (herbaceous plants) during the summer (Eastop, 1986). On the primary host eggs are laid in the autumn which hatches in the spring giving rise to wingless females which mature and give rise to several generations of wingless females asexually, by parthenogenesis. After many generations crowding and /or reduction in the host quality leads to the emergence of winged females which then disperse, in search of a suitable secondary host. The secondary host may include of plants such as weeds and vegetables. Upon finding a suitable host the winged female produces many wingless females until late summer/early autumn when a generation of winged

males and females are produced which then search for the primary host where mating and egg deposition occurs. (Blackman, 1974; Eastop, 1986; Margaritopoulos *et al.* 2006). The length of reproduction varies considerably, but averages 14 days. The average life span is about 23 days, but this depends on the condition where predators are excluded (Mackean, 2004).

The lettuce *Lactuca sativa* L. (Asteraceae (Compositae) is an important horticultural crop, widely used as source of food (Norman, 1992; Ryder, 1999). Lettuce is infected by *B. cinerea* resulting in collar rot diseases (Ramu *et al.* 2011) the infected plants develop brown necrotic lesions on the stem near the soil surface and on the lower leaves. Later the infection moves upward and finally caused death of the plant (Nikolaos and Dimitris, 2002). The aphids also affect lettuce plants causing problem worldwide resulting in economic losses (Palumbo *et al.* 1998; Palumbo, 2001). Higher aphid numbers can stunt plants, however, the most damaging effect of aphid attack is wilting and contamination of the head, which make lettuce unmarketable (Palumbo, 2001). The most common aphid pest which attack lettuce is the green peach-potato aphid (*Myzus persicae* Sulzer) (Palumbo *et al.* 1998; Blackman and Eastop, 2000; Palumbo, 2001).

The bi-directional interaction of herbivorous insect and fungal pathogens feeding on same host plant which occur only when the first attacker changes the fitness of the plant host in a way that it affects the second attacker. However, the success of the interaction is determined by the type of herbivore and pathogen involved in the interaction (Barbosa, 1991; Hatcher et al. 1995; Kruess, 2002; Cory and Hoover, 2006 Stout *et al.* 2006; Rodger *et al.* 2007).

Therefore, the present study tested two hypotheses

(i) That infestation of the plant with aphids will decrease the expression of systemic pathogen *B. cinerea* in lettuce plant.

(ii) That systemically infected plant infested with aphids will show more growth rate and high weight than plant systemically infected but uninfested with aphids.

MATERIAL AND METHODS

Experimental Plants

One hundred lettuce plants were grown, each on 15cm diameter pots filled with a vermiculite-based growing medium in a controlled environment room (18-20^{O}C, ambient humidity and 12-14 h L: D). Fifty plants were grown from uninfected seeds while the remaining fifty plants were grown from *B. cinerea* systemically infected seeds.

Plant Infestation with the Myzus persicae

Both infected and uninfected plants were infested each with ten nymphs of aphids *Myzus persicae* Sulzer (Hemiptera Aphididae) one month after germination. To allow the effect of telescoping of generation the *M. persicae* were reared on lettuce plants for three generations before being used in the experiment (Dixon, 1985). Infestation was done by placing the aphids on the reverse side of the leaves (20 infected and 20 uninfected plants) using moist brush. In order to block the escape of the aphids immediately after nymph infestation plants were covered with a vented plastic container. The remaining forty uninfested plants served as controls.

Population Size of Myzus persicae on Infected and Uninfected Plants

The population size of *M. persicae* population was obtained by counting the number of aphids on both the infected and uninfected plants. The counting was done once a week for twenty weeks, starting four weeks after infestation. Visual examination was used to assess the appearance of *B. cinerea* infection on each of the plants (Fig. 1).

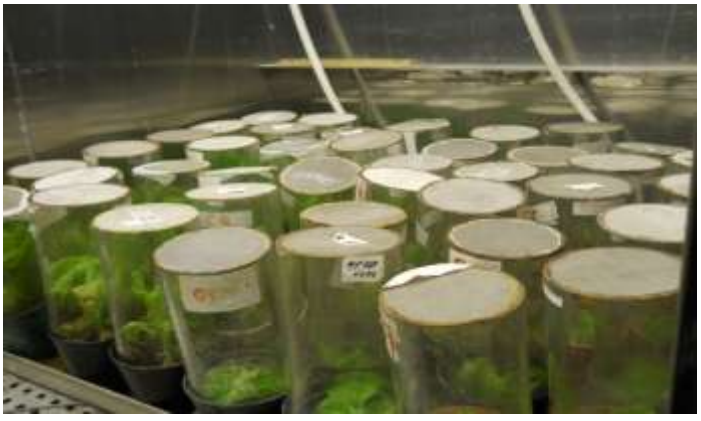

Figure 1: Infested and Uninfested Plants Grown from Infected and Uninfected Lettuce Seed in a Controlled Environment

Determination of Plant Leaf Number

Count of leaf number was taken from all 100 plants in the four treatments. Before harvest leaf number was determine by counting all the leaves from each of the experimental plants.

Measurement of Internode Length

Internode length was taken from all 100 plants in the four treatments. Before harvest length of the internode was measured from all the plants by using measuring tape.

Measurement of Plant Fresh Weight (g)

Measurement of plant fresh weight was taken from all 100 plants in the four treatments. Harvested plants were washed under running tap water and allowed to dry on a laboratory bench for one hour. Before taking the measurement, weight was measured using an electronic balance (Kern scale Technic, 440-21N).

Measurement of Plant Dry Weight (g)

Measurement of plant dry weight was taken from all the experimental plants after harvest. Complete plants were individually washed under running tap water and allowed to dried on laboratory bench for one hour before taking the measurements using an electronic balance (Kern scale Technic, 440-21N).

Statistical Analysis

Complete randomised design was adopted for the experiment. The treatments used are (a) *B. cinerea* infection status (infected/uninfected) and (b) infestation with *Myzus persicae* (infested/uninfested). The whole data was analysed using ANOVA with post-hoc Tukey tests (Hilton and Armstrong 2006). As the data from leaf number, internode length, plant fresh and dry weight did not meet assumptions of normality, therefore a Box-Cox approach was used to obtain the correct transformation before the data was analysed. The data from leaf number and internode length were log transformed while data from fresh and dry plant weight was square root transformed before the analysis. However, the differences in aphid colony number and survivorship between infected and uninfected plants was analysed using two ways ANOVA (Rehman 2013). All analyses were performed using MINITAB (2009).

RESULTS

B. cinerea Lesions on *M. persicae* Infested and Uninfested Plants

Plant infested with aphids recorded lower *B. cinerea* lesions than plants unifested by aphids. Plants uninfected by *B. cinerea* treatment were free of lesions.

Population Size of *Myzus persicae* on *B. cinerea* Systemically Infected and Uninfected Plants

Although the increase in population size of *M. persicae* on both infected and uninfected plants were slow however, the survival rate of *M. persicae* colonies was not significantly affected by plant infection status ($F_{1,99}$ = 0.65, P = 0.336). More *M. persicae* were recorded on plant free from *B. cinerea* infection, while the lowest *M. persicae* count was recorded on *B. cinerea* infected plants.

Table 1: Effect of Aphid Infestation on Plant Leaf Number and Internode Length Fresh and Dry Weight of *B. cinerea* Infected Lettuce Plants

Parameters	Plant Treatment	Test Statistics
Leaf number	*B. cinerea* infection	F1,38 = 53.72, P < 0.001
	Aphid infestation	F1,38 = 41.55, P < 0.001
	Interaction term	F1,38 = 5.29, P < 0.331
Internode length	*B. cinerea* infection	F1,39 = 63.72, P < 0.001
	Aphid infestation	F1,38 = 55.43, P < 0.001
	Interaction term	F1,38 = 4.37, P < 0.641
Plant fresh weight	*B. cinerea* infection	F1,38 = 51.22, P < 0.001
	Aphid infestation	F1, 38 = 33.41, P <0.039
	Interaction term	F1, 38 = 1.36, P = 0.334
Plant dry weight	*B. cinerea* infection	F1,38 = 71.211, P < 0.001
	Aphid infestation	F1, 38 = 53.32, P <0.001
	Interaction term	F1, 38 = 3.43, P = 0.233

Growth of Plant Parts

Effect of Systemic B. cinerea and M. persicae on Leaf Number

There was a significant effect of aphid infestation status and *B.cinerea* infection status on the leaf number of the experimental plants (Table 2) where

the presence of *B. cinerea* or *M. persicae* resulted in a significant reduction in leaf size. However, their interaction was not significant (Figure 2).

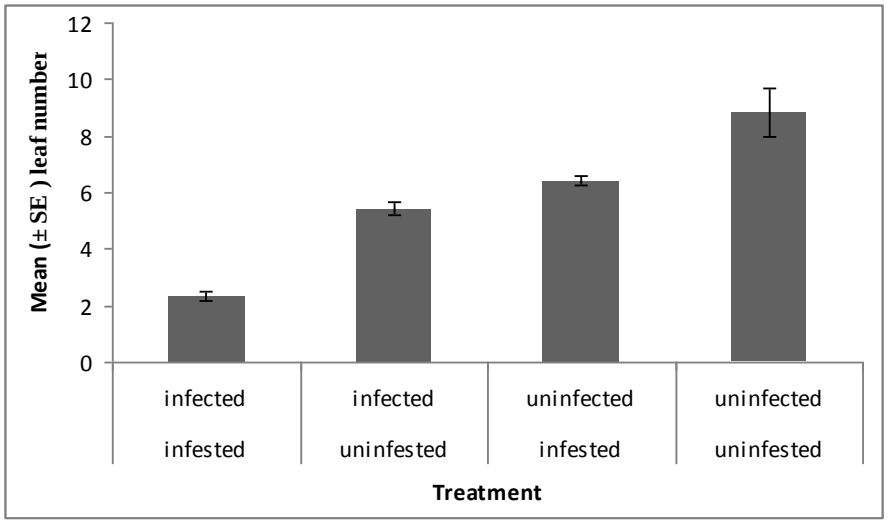

Figure 2: Leaf Number (mm) for Experimental Plants in the Presence and/or Absence of *B. cinerea* and *M. persicae*

Effect of Systemic *B. cinerea* and *M. persicae* on Internode Length

There was a significant effect of *B. cinerea* infection status and aphid infestation status on the internode length of the experimental plants (Table 1) where the presence of either resulted in a significant reduction in internode length. The interaction term was not significant (Figure 3).

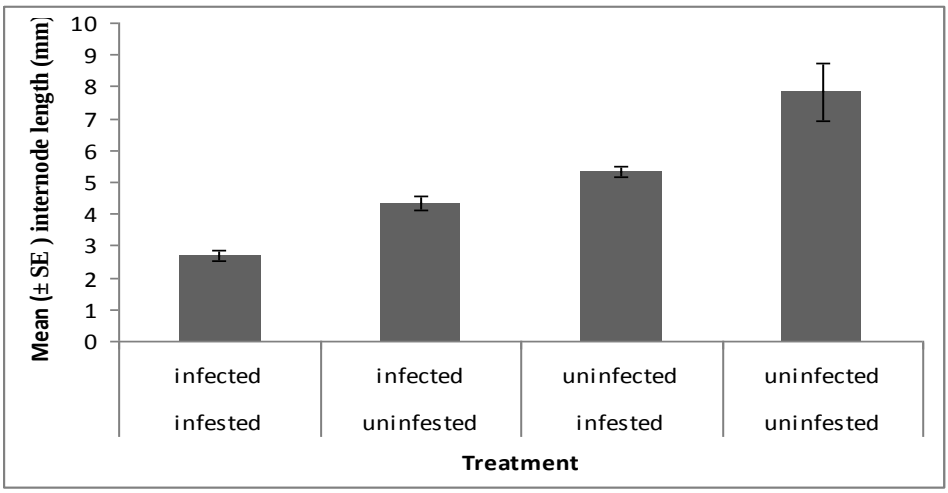

Figure 3: Internode Length (mm) for Experimental Plants in the Presence and/or Absence of *B. cinerea* and *M. persicae*

Plant Weight

Effect of Systemic *B. cinerea* and *M. persicae* on Plant Fresh Weight

There was a significant effect of *B. cinerea* infection status and aphid infestation status on the plant fresh weight of the experimental plants (Table 1) where the presence of either resulted in a significant reduction in fresh weight. The interaction term was not significant (Figure 4).

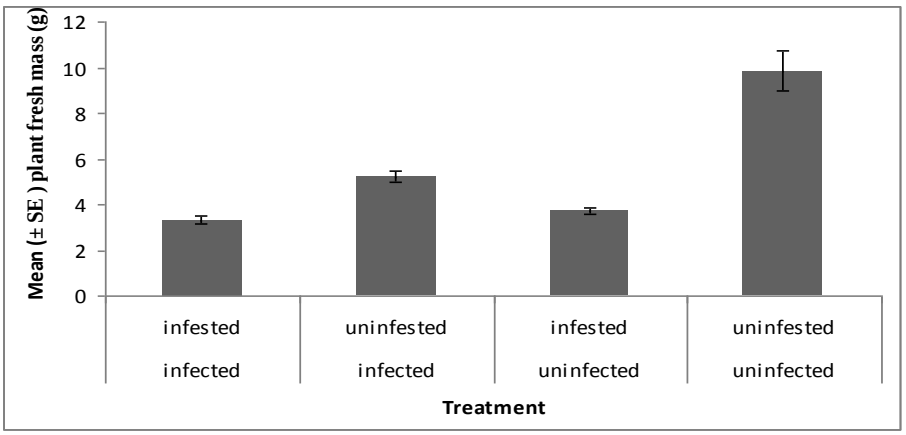

Figure 4: Fresh Mass (g) for the Experimental Plants in the Presence and/or Absence of *B. cinerea* and *M. persicae*.

Effect of Systemic *B. cinerea* and *M. persicae* on Plant Dry Weight

Infection by *B. cinerea* and infestation by *M. persicae* or resulted in a significant decrease in plant dry weight (Table 1). The interaction term was significant (Figure 5).

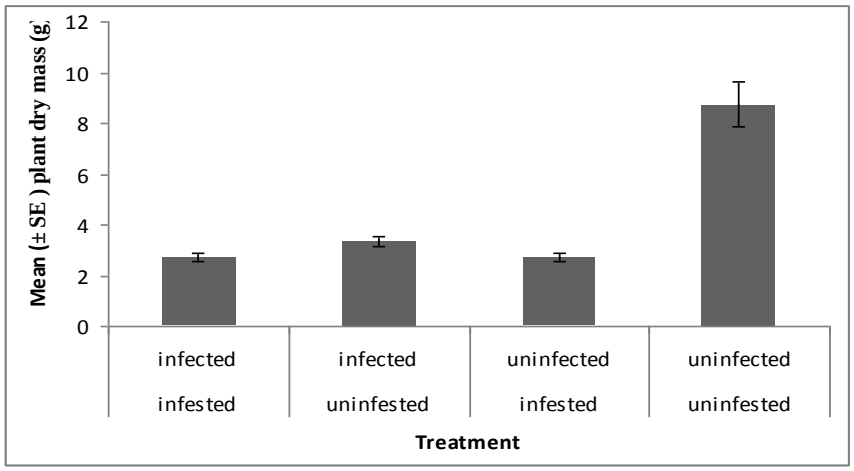

Figure 5: Dry Mass (g) for the Experimental Plants in the Presence and/or Absence of *B. cinerea* and *M. persicae*

DISCUSSION

The present study enable us to identify a bi-directional interaction between herbivorous insect *M. persicae B. cinerea* a pathogenic fungi. Rates of expression of *B. cinerea* lesions were lower on infected plants when infested with aphids; also aphid numbers were lower on the infected plants. Fresh and dry weight of the plants was lowest in *B. cinerea* infected plants which were not infested with *M. persicae*. Lettuce plants systemically infected by *B. cinerea* showed reduced leaf number, internode length more than systemically infected plants which were infested with aphids. In a related experiment Rostas (2002) reported that these types of interactions potentially impact on the life history traits of the attackers, such as herbivore performance or growth of pathogenic fungi and thus could be among the leading factors in terms of herbivore and pathogen population dynamics.

The present study fully established that leaf number, internode length, fresh and dry plant weights were significantly reduced by individual effect of *M. persicae* or *B. cinerea* attack. However, when both are sharing the same host plant only the dry weight was decrease significantly but leaf number, internode length, fresh weight were not significantly reduce. This indicates that appropriate method for the control of *B. cinerea* is needed to reduce losses of plants ant its produce.

Agrios (2005) reported that reducing economic losses due to *B. cinerea* infection can be achieved by the use of chemicals for treatment of seeds, fumigating soil, spraying plants or the post-harvest treatment of crops (Agrios, 2005). However, chemical treatments of *B. cinerea* were estimated to have cost about 540 million Euros worldwide in 2001, which represents 10% of the world fungicide market (UIPP, 2002). Apart from the rising cost of production, chemical treatment of *B. cinerea* adds toxic chemicals to the environment which becomes a health hazard and limits the land available for cultivation due to contamination (Agrios, 2005). Therefore dramatic research efforts were focused on management of the fungus using environmentally friendly strategies such as biocontrol using insects herbivores (Pal and Gardener, 2006; Pscheidt, 2007). However, the success of biocontrol largely depends on the level at which the fungal pathogen and insect influence each other (Moran and Porath 1980).
The present study found that expression of *B. cinerea* was reduced after infestation with an insect *M. persicae* when compared to non-infested plants. This finding was similar to the results obtained by Mouttet *et al.* (2011) who

showed that infestation of *B. cinerea* infected plants with aphids (*Rhodobium porosum* Sanderson) courses lower expression of *B. cinerea* and the infestation triggers the plants to induce Salicylic acid (SA)-dependent pathway around the infection site which kill *B. cinerea* cells and stop it growth. Although, continuous feeding on cell contents by the aphids triggers the plant to induce the wound-response pathways, (JA) and (ET) dependent pathways which reduce the population size of aphids (Kanno and Fujita, 2003; De vos, *et al.* 2006; Hatano *et al.* 2008; Wassim *et al.* 2008).

In a related experiment, with plant host, Brussels sprouts, Heng-Moss *et al.* (2003) reported that the reduction in the plant fresh and dry weight, leaf number and internode length occurs due to the reduction of photosynthesis in leaves which have been injured by the pathogen attack due to an increased synthesis of defensive chemicals in response to the attack. In another experiment using Brussels sprouts Delucchi (1976) reported that plants which where subjected to probing and salivation by *M. persicae*, and on which continued feeding by the aphid was prevented, showed a similar reduction accompanied by increased respiration of the plant. In agreement with the present study Delucchi (1976) and Mackauer and Way (1976) concluded that the increased respiration of the plant, a response to either pathogen or aphid wounding/salivation, contributed considerably to a reduction plant fresh and dry weight plant height, leaf size and number. From the present study it was clear that this occur because they assimilates which should have been use by plant and excess made available for storage are continuously been use to provide defence against the either *B. cinerea* or *M. persicae* attack. However, when both *B. cinerea* and *M. persicae* are sharing the same host plant they tend to reduce the effect of each other through the secretions of chemicals which make the host plant unfavourable for the feeding and growth of each other.

This research has confirmed that aphids infestation on *B. cinerea* infected plant reduce the expression of the pathogen by the plant. In general, the results of the experiment show that growth of *B. cinerea* is affected by the presence of aphids on the plant. Moreover, the experiment confirmed the existence of a negative relationship between *M. persicae* and *B. cinerea* where they independently stress the host plant, but opposed each other when sharing the same host plant by triggering the induction of defence chemicals by the plant at the expense of other vital functions. Although, infection of *B. cinerea* is reduce by the presence of aphids on the plant however, aphids a known to transmit viral

diseases therefore further research need to be carried out between ecologist and plant pathologist to determine on how aphids could be use for the biocontrol of *B. cinerea* without causing viral infection to the plants. Therefore this research is an excellent valuable pointer in increasing our understanding of the ecological consequences of a ubiquitous but hitherto understudied interaction.

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